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MICROSURGICAL INSTRUMENTS AND ATTACHMENTS

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MICROSURGICAL INSTRUMENTS AND ATTACHMENTS

[Following is the translation of an article by A. B. Lange, Entomology Department, Moscow State University, published in the Russian-language periodical Zoologicheskii Zhurnal (Zoological Journal), 1963, Vol. XLII, issue 8, pp 1257-60. Translation performed by Sp/6 Charles T. Ostertag Jr.]

For the delicate preparation of insects, ticks and other minute invertebrates, miniature surgical instruments are used which are usually prepared by the experimenter himself. In this article we will share the experience of preparing and using several special instruments and attachments.

Microsurgical Instruments on the Basis of Surgical Tweezers

For preparing minute objects it is often necessary to use microsurgical instruments with two working ends: Tweezers, forceps, scissors, and others. In particular, microforceps are used when extracting hormone organs in insects (Thomsen, 1942). The construction of microscissors is described in the work of A. A. Makhotina (1955). The main condition for the successful working of such instruments is the maximum precision of convergence of the end sectors. As the basis for microsurgical instruments we used small surgical tweezers which had on the ends a triangular protrusion and a recess which are used as guides and guarantee the precise convergence of the ends of the instruments. On the outside towards the ends of the tweezers are soldered cuttings of steel wire of the necessary gauge, entomological pins or insect pins, the ends of which are then formed depending on the type of instrument: Ground off bluntly in the form of tweezers (fig 1,1), forged, bent inwards and ground in the form of forceps (fig 1,2), bent at an angle and pointed for making scissors (fig 1,3), etc. It is more convenient to make the latter with a reverse working movement - the convergence of the tips when the tweezers are open, which eliminates the necessity of manual pressure in the working moment. A transverse, wire rib-stop is soldered on the tweezers. By lightly bending the rib, one can obtain any degree of opening of the tweezers in the open position. In practice the free opening of the ends of a microinstrument in various cases varies from 0.2 up to 1.5 mm. [Evidently an error in the text.]

The preparation of microsurgical instruments on the basis of surgical tweezers requires technical skill. All the operations, soldering, forging,

grinding the ends of the instruments, their tempering, sharpening, and setting are carried out according to the generally applied technical rules, but due to the miniature size of the instruments, under a binocular.

Stand for Fixation and Constriction of an Object Being Operated On

When operating on minute live objects the usual method of fixing with needles in a wax bath is not convenient. Besides this, it is often necessary to not only fix the object but also to make a constriction of individual parts of the body. For this aim we constructed a stand, the detailed layout of which is presented in figure 2. The stand is made out of metal (more preferably out of brass). On the stand there is a frame (2) on which a detachable strip (3) made out of plexiglas is fitted tightly. Detachable strips are made of a various width and with profiles which correspond to the objects (fig 2,B). The object is fixed on the strip with the help of an elastic band made of a synthetic film (chlorovinyl, polyethylene and others) spanning across the strip. A window is cut in the band and the operation is performed through it. The thickness of the film, the width of the band and the form and dimensions of the window are selected to correspond to the dimension of the object, its consistency and the missions of the operation (fig 2,V). Tension of the band is regulated by a screw (5) which is connected with a sliding plate (6). On the end of the plate is a clamp with a screw (7) for securing the ends of the band. The band is stretched over a roller (8) and passes between the guiding plates (9) which eliminate misalignment during tension. The stand is fastened by screws on the stage of the binocular dissecting guide.

Attachment for Fixing an Object on a Microscope Slide

Minute objects must often be fixed on a microscope slide in order to be dissected in passing light and also so that without extracting individual organs it is possible to examine them under a microscope, in particular with the help of water immersion lens, submerging it into the dissected object which is filled with a physiological solution.

For this purpose we used sliding tension members with catches (fig 3). The tension members are triangular plates made of plexiglas or glass on the ends of which are fastened catches made out of entomological pins or iron pins. The plates are fitted to the slide in vaseline oil and can be moved in a horizontal plane. The catches are bent under in such a way that when pushed up the plates they rest with the ends in the slide. Before dissection, the object is fastened with two tension members and subsequently during the operation the edge of the section and individual organs are drawn to the sides by other tension members.

Micropipettes

For the injection of liquids and the transfer and implantation of small

organs, fine pipettes are used. In a number of cases the diameter of the working channel is 0.1 to 0.01 mm and the length up to 10 mm. Such micropipettes can be manually drawn out in a gas microburner from glass tubes with a diameter of 4-6 mm without resorting to more complicated methods recommended in working with a micromanipulator. The main condition for the successful working of a micropipette is the even feeding of a liquid which is usually accomplished with the help of syringes of various construction, special pumps, mercury pumps, etc. (Fonbrune, 1961, and others). For transferring small organs extensive use is made of a pipette with a neck which retains the object in the end portion of the working channel (Ephrussi et Beadle, 1936).

We constructed and successfully used a micropipette with end pressure which is prepared in accordance with the plan in fig 4. Such a pipette ensures an even feeding of liquid and retains an object in any part of the working channel. The liquid is fed by pressure of the plate (2) made out of vacuum rubber on the rear section of the pipette, which is smoothly soldered along the edges. Between the rubber and the pipette is interlayered an elastic synthetic coating (3) since under the considerable pressure which is achieved in such a pipette the rubber soaks up the liquid and disrupts the even feeding. The feeding of the liquid is carried out with a screw (4) which presses on the sliding metallic holder of the rubber plate (5). The pipette is located in a detachable holding device (7) which is secured by a screw (8). The bottom of the holding device serves as an arresting device for the pipette. The tapering part of the pipette protrudes through an opening in the bottom of the holding device. Before operation, the holding device with the pipette is removed and the pipette is filled with the liquid (with the help of another fine pipette with the usual rubber cap). Then, the holding device with the pipette is placed in position and the pipette is pressed with the rear section to the feeding device so that no air bubbles remain inside and the holding device is secured by the screw. The feeding screw is turned up until the appearance of a trickling of liquid on the end of the pipette. When working with very thin pipettes, it is more expedient to fill them with vaseline oil, thereby accumulating the required volume of introducible liquid in the end part of the working channel.

The metallic base with the pipette and feeding device is attached on a special stand (fig 5,A). Shifting and fixing the pipette in the required position is ensured by screw clamps: screw 1 - longitudinal motion and horizontal turning; screw 2 - vertical inclination; screw 3 - putting into and taking out of the working position and fixation; screw 4 - adjustment of a permanent working level during numerous repetitions of the experiment; screw 5 - fixing the stand at the necessary height. The stand is attached by a sleeve with a screw on a vertical shaft which is mounted on the stage of the microscope or binocular or on the dissecting guide. In the first case, the object is fixed on the dissecting stage with a four way movement and moved to the pipette; in the second, the pipette is moved to the object.

For the most delicate operations a manual pipette has been prepared on the basis of outside calipers (fig 6,A). The needles and their supporting screws are removed from the outside calipers and on its ends there are soldered two metallic plates with bent edges which envelop the rubber tube of the pipette. The screw on the outside calipers serves for feeding the liquid. The thickness of the walls of the rubber tube is 2-3 mm, its rear end is closed by a fragment of a soldered glass rod. When ready for operation the pipette and rubber tube are filled with the liquid separately with the help of another fine pipette (fig 6,B) and joined together in such way that no air bubbles remain; the tube is enclosed between the plates of the calipers and compressed by the screw until the appearance of a trickling of liquid on the end of the pipette.

The pipette with the rubber tube may also be attached to the stand described above. For this an additional removable attachment is made (fig 5, B) in which the pipette is made fast by a screw (1) and the liquid is fed by pressure on the lateral plate with a screw (2).

The instruments and attachments described make it easy to conduct very fine operations: Dissection of live objects of an order of 0.3 - 0.5 mm, in our practice the embryos of Arthropods and others, the extraction and implantation of small organs, for example, hormone glands of insects (Tamarina, 1963), the injection of hemolymph, dye solutions, etc.

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[The following English summary appears with the Russian original.]

Instruments and devices made and applied by the author for the preparation of small Arthropods are described: microsurgical instruments on the basis of surgical pincers, mounting for the fixation and ligature application on the subject operated, an adjustment for fixing the subject on the slide, micropipettes.

Figure 1. Microsurgical instruments on the base of surgical tweezers.
1 - tweezers, 2 - forceps, 3 - scissors.

Figure 2. Stand for fixation and constriction of an object being operated on.
A - lay out of the stand: 1 - body, 2 - frame, 3 - detachable strip, 4 - band, 5 - screw for regulating the tension of the band, 6 - sliding plate, 7 - clamp for securing the ends of the band, 8 - roller, 9 - guiding plates; B - set of strips made of plexiglas; V - variations of the working aperture in the band.

Figure 3. Attachment for fixing an object on a microscope slide.
1 - microscope slide, 2 - tension members with catches, 3 - vaseline grease,
4 - object.

Figure 4. Lay out of micropipette with end pressure (cross section).
1 - micropipette, 2 - rubber plate, 3 - film, 4 - screw for feeding liquid,
5 - sliding holder, 6 - base, 7 - holding device, 8 - screw fixing the tube
with the pipette.

Figure 5A. Stand for the pipette fastened on the stage of the binocular. Fixing screws for: 1 - the longitudinal motion and horizontal turning, 2 - vertical inclination, 3 - putting into and taking out of the working position, 4 - adjustment of working level, 5 - feeding of liquid, 6 - sleeve with screw for attaching stand on the stage of the binocular; B - Detachable attachment for the pipette with a rubber tube: 1 - screw fixing the pipette, 2 - screw for feeding of liquid, 3 - screw for the vertical slant of the pipette.

Figure 6A. Manual pipette on a base of outside calipers, B - pipette for filling with liquid.